PIGMENT INHERITANCE IN THE FUNDULUS–SCOMBER HYBRID
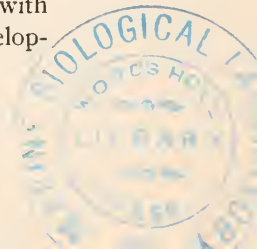
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The hybrid between *Fundulus heteroclitus* (L.) ♀ and *Scomber scombrus* (L.) ♂ is first mentioned by H. H. Newman in 1915. J. Loeb had stated his belief that development in intergenic hybrids is partheno-genetic. Newman cites the inheritance of *Scomber* pigmentation in the *Fundulus-Scomber* cross as a proof that fertilization had taken place. In a later paper, 1918, he continued the discussion of this hybrid.

His account of the abnormalities found in the embryos made it seem worthwhile to obtain the cross again, to make a cytological and mor-phological study of early stages, and to make a detailed study of the chromatophores in parent and hybrid embryos.

Preliminary hybridizations were made successfully late in June, 1937, at the Marine Biological Laboratory. During the summers of 1938 and 1939 numerous hybridizations were made from June 10–July 5. After July 5 it is usually impossible to procure spawning mackerel, before June 10 it is difficult to obtain spawning *Fundulus*. For best results, with a large percentage of hybrid embryos, both parents must be at the height of sexual activity.

Ripe *F. heteroclitus* females were selected and isolated in tanks of running sea water for at least 18 hours before they were to be used: this assures the absence of *Fundulus* sperm. As mackerel do not live long after being caught, hybridizations were carried out at the traps. *Fundulus* females were carried to the fish traps in clean buckets or bowls of sea water. *Scomber scombrus* males were stripped into finger bowls containing a small quantity of sea water. The *F. heteroclitus* females were stripped into the sperm suspension, the eggs from each female being kept in separate bowls. After 10–15 minutes the sperm suspen-sion was washed off and fresh sea water was added. In the laboratory the eggs were placed a few in each bowl, and allowed to develop. Con-trols were carried as follows: (1) Unfertilized *F. heteroclitus* eggs from some of the females in each set of hybridizations were observed as a check on the possible presence of *F. heteroclitus* sperm in sea water, in the tanks, or on the fish. (2) *F. heteroclitus* eggs were fertilized with *F. heteroclitus* sperm to check on the fertilizability and rate of develop-

ment of normal *Fundulus heteroclitus*. (3) *Scomber scombrus* eggs were fertilized with *Scomber scombrus* sperm to check on the normal *Scomber scombrus* development. (4) The reciprocal cross with *Scomber scombrus* eggs and *F. heteroclitus* sperm was tried many times, always unsuccessfully.

The egg of *Fundulus heteroclitus* is 2–2.5 mm. in diameter, well yolked, demersal, developing slowly and hatching out in 12–16 days. The egg of *Scomber scombrus* is smaller, 1 mm. or less in diameter, transparent, pelagic, developing quickly and hatching in 60–72 hours. The hybrid develops more slowly than normal *F. heteroclitus*, forming defective embryos which, in our experience, never hatch, even though kept for 30–35 days.

Normal stages of *Scomber scombrus* have not been described, although Worley (1933) mentions that they resemble closely those of sea bass as described by Wilson (1889). As there was no published account of pigment development in *Scomber scombrus,* this had to be studied. *Scomber scombrus* eggs were obtained and fertilized at the fish traps. As the eggs are pelagic, it is difficult to wash off the excess milt while in transit from traps to laboratory; sea water can, however, be added from time to time. *Scomber* eggs are very sensitive to temperatures above 17° C., and will not develop at all above 21° C. (Worley, 1933), therefore care must be taken that the water in the shallow bowls is not warmed by the sunshine, or by heat from the decks or engine. In the laboratory the fertilized and developing eggs soon float to the top of the water, and can be skimmed off and transferred to fresh sea water. If the bowls are placed in baths of running sea water the eggs develop quite normally, and hatch out in 60–72 hours. The first pigment cells to appear are the slender branching melanophores on the dorsal surface of the embryo at 27 hours. Later more melanophores appear and form the characteristic pattern; a band across the dorsal surface of the head at the level of the optic vesicles, and a row along the lateral line region. A few migrate to the yolk sac and to the oil drop. At 36 hours there appears just behind the optic vesicles a group of cells containing yellowish green pigment granules. Soon the granules increase in number, the pigment cells fuse, forming two large brilliantly green chromatophores persisting at least as long as the fry live in the laboratory. Other green chromatophores may appear behind the otic vesicles, on the oil drop or near Kupfer's vesicle. Upon hatching the young fry drop to the bottom of the vessel and lie there until they are able to swim about easily.

Normal stages of *Fundulus heteroclitus* have been described (Oppenheimer, 1937). Typical pigment formation has been described, also

(Bancroft, Stockard, Newman).   Four days after fertilization a first head crop of melanophores appears.   Another crop appears on the fifth day.   The pigment cells are of three types: those on the yolk are large polygonal melanophores with but few processes, those on the embryo are smaller and more branched: the reddish-orange much-branched chromatophores found on embryo and yolk sac.   On the sixth day, when circulation begins, the melanophores of the yolk sac migrate to the blood vessels and fuse.   The reddish-orange chromatophores also arrange themselves along the course of the blood vessels, but do not seem to fuse.   A number migrate to the lateral line.   Melanophores are rarely seen in this region, the absence of a visible lateral line being one of the species characteristics of the adult *F. heteroclitus.*   After hatching few reddish chromatophores are to be found on the exterior of *F. heteroclitus.*

The melanophores of *F. heteroclitus* and *S. scombrus,* the green chromatophores of *Scomber,* and the reddish chromatophores of *Fundulus* all contain a granular pigment.   No green chromatophores are ever present in *Fundulus heteroclitus,* and no red chromatophores in *Scomber scombrus.*

Fundulus-Scomber hybrids cleave at the same rate, or more slowly, than normal *Fundulus heteroclitus.*   In a series of 15 hybridizations, 3,097 eggs were fertilized: 3,084, or 99 per cent of these cleaved. Many died at gastrulation and during early embryonic life, but 1,205 or 39 per cent formed advanced embryos.   Development in the hybrid is slower than in normal *F. heteroclitus:* pigmentation develops later, the heart does not begin to pulsate as early, circulation is feeble, or not established in most of the hybrids.   As a result, the chromatophores remain scattered for a longer period, eventually migrating to the heart, or to the site of its attachment to the yolk.   As has been noted by Bancroft, Newman and others, the hybrid embryos show various combinations of the parental types of chromatophores.

Figure 1 *a* shows the average melanophore counts for parent and hybrid embryos.   As their rate of development differs widely stages which are equivalent were arbitrarily chosen.   Normal *F. heteroclitus* five days after fertilization shows both first and second crops of melanophores not as yet fused on the blood vessels.   *Scomber scombrus* at 30 hours shows first and second crops of melanophores also, not yet migrated to head and lateral line regions; the hybrids at 7 days showed the melanophores well developed, not yet fused.   *F. heteroclitus* embryos show from 8–18 melanophores, or an average of 13—on the dorsal surface; *S. scombrus* has from 34–51, averaging 40.   The *Fundulus-Scomber* hybrid shows a great variation, from 2–44.   The majority of
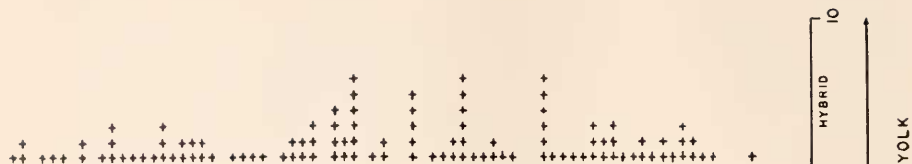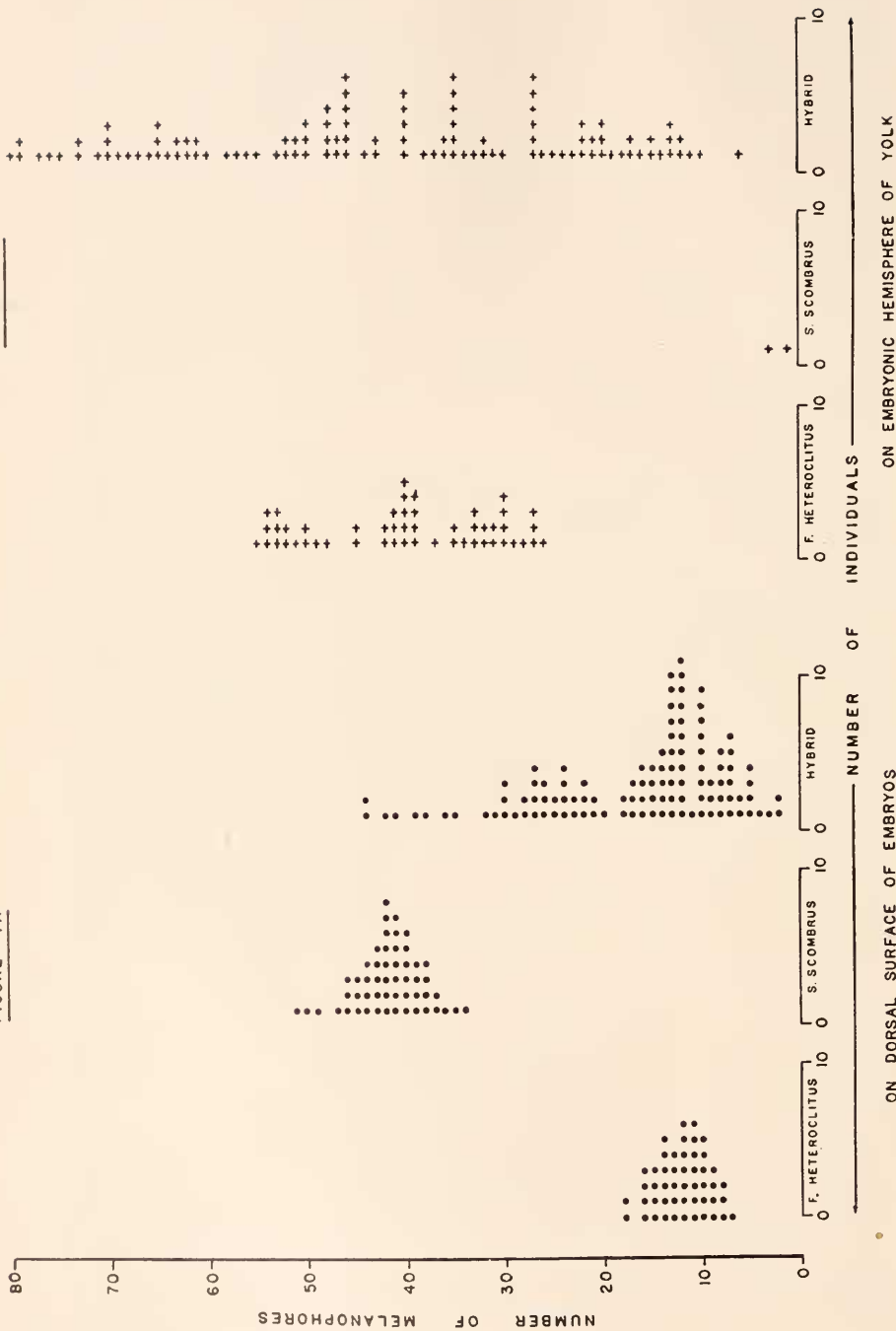
DISTRIBUTION OF MELANOPHORES IN PARENT AND HYBRID EMBRYOS

Fig. 1. *A* and *B*. Distribution of melanophores in parent and hybrid embryos.

hybrid embryos seem to show a tendency to conform to the *F. hetero-clitus* type of distribution, having 8–18 melanophores on the dorsal surface of the embryos. However, we have seen no normal *F. heteroclitus* embryos of 5 days with over 20 melanophores—the increase in number of melanophores present in a large number of hybrid embryos may be assumed to be the influence of *Scomber scombrus*.
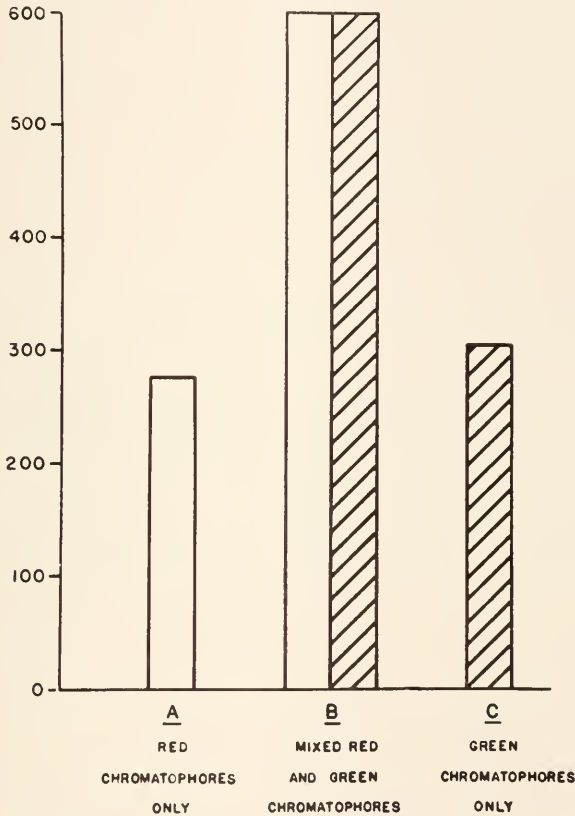
CHROMATOPHORE INHERITANCE IN THE HYBRID EMBRYOS

Fig. 2. Chromatophore inheritance in the hybrid embryos.

Figure 1 *b* shows the distribution of yolk melanophores, counted on the embryonic hemisphere of the same individuals used above. In *F. heteroclitus* the yolk melanophores are large polygonal cells, varying from 26–55 in 5-day-old embryos. *Scomber scombrus* at 30 hours rarely shows any yolk melanophores. In the 7-day-old hybrid embryos the number varies from 6–80, with scarcely two embryos having identical numbers of melanophores.

The hybrids can be grouped roughly into three categories: those showing green chromatophores of the *Scomber* type, but no red *F. heteroclitus* type chromatophores: those showing the red *Fundulus* type, but no green *Scomber* type chromatophores: those showing both red and green chromatophores. Figure 2 will show the distribution of 1,205 ten-day-old hybrid embryos in these three categories. There seems to be a very significant relation at first glance, since about equal numbers of individuals show only maternal or paternal type chromatophores, and about twice as many show both types combined. As a matter of fact, examination shows that no two embryos are identical. In the group showing green chromatophores only, the individuals range from those brilliantly green laterally to some with only a few green head chromatophores: some show green chromatophores on the embryo only, some show them on the yolk also. In the group showing only red chromatophores of *Fundulus* type, the individuals are equally variable, some embryos being brilliantly reddish-orange, others grading to some resembling closely *F. heteroclitus* embryos. In the large group showing both red and green chromatophores there is every conceivable type of combination, no two individuals are identical. In addition, the *Scomber* and *Fundulus* types of melanophores are present in all possible combinations with the red and green chromatophores, in all three categories. No consistency of pigment distribution is found in sets of *Fundulus* eggs from various females, fertilized at the same time by sperm from the same mackerel: some may show a preponderance of green, of red, or of mixed green and red chromatophores as will be seen in Table I.

TABLE I

Green Chromatophores Only	Mixed Red and Green	Red Only
84	57	43
27	41	3
27	107	35
2	29	21
2	12	29

These counts were made on eggs from various *F. heteroclitus* fertilized by sperm from the same mackerel on June 17, 1939, and counted June 27, 1939.

Unlike Newman, who reports that green chromatophores can be found only in hybrid embryos obtained before mid June, we have found green chromatophores in the hybrids whenever we got successful crosses.

DISCUSSION

Many intergenic and interspecific crosses have been described. Some of these "hybrids" do not develop beyond late embryological

stages, as is true of the *Fundulus-Scomber* hybrid we are discussing. Here we are dealing with a much wider cross, for the two genera belong to different sub-orders. They differ widely in ecological relations, in habitat, and in structure. Morphological studies of hybrid and parent embryos may show significant combinations of structural peculiarities, for example: *F. heteroclitus* has an air bladder, while this structure is absent in *Scomber scombrus*. Cytological studies may show successive elimination of chromosomes during early cleavages. It is probable that some of the embryos showing only *Fundulus* type chromatophores, and typical *Fundulus* distribution of melanophores, may be haploid individuals. These individuals are rare, however, for even in this group one generally finds some *Scomber* type melanophores, or some *Scomber* effect on the number or distribution of melanophores. (Figure 1, *a* and *b*.)

It is probable that in the teleosts, as in the amphibians, pigment differentiation depends on neural crest development. If so, it is reasonable to suppose that wherever *Scomber* type chromatophores are present, *Scomber* chromosomes may have been retained throughout cleavage, gastrulation and differentiation.

We have purposely refrained from a discussion of the size of melanophores and chromatophores in parents and hybrids, for any measurements would be open to the criticism that metabolic processes in the embryo are abnormal, or at least, much disturbed. In shape, the melanophores of *Fundulus* are quite distinct and distinguishable (Newman and others). In color the reddish chromatophores in the hybrids are identical with those of *Fundulus heteroclitus,* the green chromatophores identical with those of the *Scomber* parent.

In the literature on interspecific and intergenic crosses, the F_1 generation is generally reported as intermediate. A closer scrutiny of the hybrids may reveal a much wider variation than hitherto suspected, as, for instance, in the case of the melanotic hybrids between *Platypoccilius* ♀ and *Xiphophorus* ♂ described by Gordon. Apparently a case of Mendelian dominance is revealed in the universally melanotic F_1 generation, but there is reported a variation in degree of melanosis in the progeny.

Earlier reports (Newman, Bancroft) have attempted to explain the results of the *Fundulus-Scomber* hybridizations on the basis of Mendelian dominants and recessives, or of " blending " inheritance. However, as no detailed study of pigmentation was attempted, the enormous variability actually present in the hybrids escaped attention.

Pinney, reporting on other inter-sub-order hybrids, reports chromosome elimination during early cleavages.

We have at this time no satisfactory explanation for the phenomenon presented by the pigment inheritance in this cross, although we may assume that there is a complicated random assortment, combination and elimination of chromosomes.

We are indebted to Mr. Robert Goffin of the U. S. Bureau of Fisheries, at Woods Hole, and to the crew of the " Sagitta " of the Marine Biological Laboratory for their assistance in getting to the fish traps and obtaining mackerel. We wish also to thank Dr. H. B. Goodrich, for his interest, and also Rev. F. W. Ludwig, Ph.D., for help with the microphotographs.

Summary

1. Methods for hybridizing *Fundulus heteroclitus* and *Scomber scombrus* are described.

2. The pigment development in hybrid and parents is described.

3. Comparison of the inheritance of embryo and yolk melanophores reveals a *Scomber*-effect in the embryo.

4. The hybrid embryo shows chromatophore inheritance from both parents.

5. Actually, as regards inheritance of melanophores and chromatophores, there is enormous variability, no two embryos being identical as to pigment distribution.

6. This variation in the F_1 generations is unusual and at present inexplicable.

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